Problems and Prospects of Rhizoctonia Diseases and Their Control in Japan

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摘　　要

Ui, T. (1985) 日本 **Rhizoctonia** 病害之問題與前瞻及其防治　（日本北海道大學）

日本產 *Rhizoctonia solani* 可分爲 8 個菌絲融合羣 (anastomosis group, AGs)，部分融合羣並具有亞羣 (subgroups) 。各 AGs 之菌株是以DNA-DNA 再配對動力學 (DNA-DNA reassociation kinetics) 之方法研究其遺傳純一性 (genetical homogenicity) 。具植物病原性的 Rhizoctonias 屬於 AG 1-5，這些菌株均由北海道的耕地及未耕地的植物及土壤中分離而得。由 *R. solani* 引起的土壤傳播性病害遍佈日本，如各類植物的猝倒病、馬鈴薯的黑痣病、甜菜的根病等。雖然有 10種以上之藥劑被用以防治 Rhizoctonia 病害，但除非使用燻蒸劑，否則難以完全根除土壤中的接種源。

本報告討論 *R. solani* 的生物學及以紙盆種植甜菜直接移植方式 (paper pot transplanting system) 防治 Rhizoctonia 病害之可行性。AG 4 及 AG 1-B 爲引起幼苗猝倒病的病原菌，可以 PCNB 混於紙盆的上層土中而加以防治。AG2-2 引起的根腐病在盛夏發生，以殺菌劑施於根頸 (crown) 可達到防治效果，而以 PCNB 及滅普寧 (mepronil) 最爲有效。AG 2-2 的擔孢子可引起葉枯病，噴洒滅普寧及依普同 (iprodione) 殺菌劑可有效予以防治。紙盆移植方式廣爲採用後，甜菜 Rhizoctonia 病害尤其是根腐病顯著的減少。此法不但可更經濟有效的使用化學藥劑，而且可大大減少土壤中接種源的密度。

由 AG 2-2 引起的根腐病在甜菜連作田中初期發病嚴重，隨後病勢減輕。此種能使病害減少的土壤對 AG 2-2 有顯著的抑制效果。因此紙盆中加入此種土壤可能是有效的生物防治方法。

ABSTRACT

Rhizoctonia solani in Japan is divided into 8 anstomosis groups (AGs), and some of them include subgroups. The genetical homogenicity in the isolates of respective AGs has been studied by DNA-DNA reassociation kinetics. Plant

pathogenic Rhizoctonias belong to AG 1-5, and they are isolated from the plants and soils in cultivated and uncultivated areas in Hokkaido. The widely distributed soil-borne diseases caused by *R. solani* in Japan are damping-off various plants, black scurf of potatoes, root rot of sugar beets, etc. More than ten fungicides have been registered for the control of Rhizoctonia diseases, however, complete eradication of soil inoculum is difficult except by fumigants.

In this report biology of *R. solani* and control of Rhizoctonia diseases of sugar beet grown under paper pot transplanting system were discussed. AG 4 and AG 1-B are the pathogenic AGs to cause seedling damping-off. They are controlled by PCNB mixed with upper layer of paper pot soil. Root rot occurring in midsummer is caused by AG 2-2 and it is controlled by the spray of fungicides to crown. PCNB and mepronil are generally effective. Basidiospores of AG 2-2 cause the foliage blight. Spray of fungicides, such as mepronil and iprodione is most effective. Rhizoctonia diseases of sugar beet, especially root rot decreased remarkably, after the paper pot transplanting system was widely adopted. The system makes it possible to apply chemical control economically and effectively, and the inoculum level of *R. solani* in soil can be greatly reduced.

The root rot caused by AG 2-2 declines, after an initial severe outbreak, during monoculture of sugar beet. The disease declined soil is remarkably suppressive to AG 2-2, and it might be possible to use such soil in paper pots for effective biological control of the disease.

A brief history of soil-borne diseases and their control in Japan

Since the 17th century, Japan's agriculture has progressed rapidly centering around the cultivation of rice. Barley, wheat, rape and some vegetables were cultivated as second crops to rice in paddy field after drainage of the soil. It is probable that there were few serious problems of root diseases of upland crops grown under such conditions, because rapid buildup of soil-borne pathogens in the soil must have been prevented by repeated flooding and drainage. In the 18th century, the intensive cultivation of tobacco, cotton, and vegetables in the central parts of Japan was attended by the increase of soil-borne diseases. In the agricultural classics published in those days, there were several descriptions on the symptoms of soil-borne diseases attributed to soil-sickness or inadequate crop sequences, and long term rotation with different crops was recommended.

Japan's plant pathology dates from the last decade of the 19 th century, and diseases of rice had been the most important research subjects until 1953 when organic mercury fungicides achieved a great success in the control of rice blast. During those days, research on soil-borne diseases was carried out sporadically. There were two epoch-making control techniques: control of Fusarium wilt of watermelon by resistant

rootstocks reported by Murata and Ohara in 1936[12], and control of Cephalosporium stripe of wheat by flooding of wheat field reported by Ikata and Yoshida in 1937[5].

Another interesting method was the application of Trichoderma spores into soil, From studies on the antagonistic action of microorganisms carried out by Nishikado and associates during the 1940s, "Trichoderma Dust" was developed. The dust developed by Ohshima, one of Nishikado's associates contained spores of *Trichderma lignorum* (10^8/g) and was registered in 1956 for the control of stem rot of tobacco caused by *Sclerotium rolfsii*.

Anastomosis groups of *Rhizoctonia solani* in Japan

In the extensive studies on the physiological specialization of *R. solani*, Matsumoto[11] concluded in 1923 that "the fusion of hyphae may take place only between the hyphae from strains which are closely related" and later he pointed out that the hyphal anastomosis can be used as a criterion for the recognition of *R. solani* and *Corticium sasakii*. Hyphal anastomosis is now widely accepted in the grouping of *R. solani* and eight such anastomosis groups (AGs) have been recognized in Japan (Table 1). From the

Table 1. Anastomosis groups of *Rhizoctonia solani* found in Japan

Anastomsis[1] Group	Subgroup	Ecological group[3]
AG-1	I-A[3]	sheath blight type
	I-B	web blight type
AG-2-1		winter crop type
AG-2-2	IIIB[3]	rush type
	IV	root rot type
AG-3		potato type
AG-4	HG-I[5]	praticola type
	HG-II	
AG-5		
AG-6	HG-1[5]	
	GV	
AG-7[2]		
AG-BI[4]		

1) Ogoshi (1976) 2) Homma et al (1983)
3) Watanabe & Matsuda (1966) 4) Kuninaga et al (1978)
5) Kuninaga & Yokosawa (1984)

evidence on DNA base composition of *R. solani*, each of the AGs is considered to be genetically independent unit[9].

Some AGs were each divided into subgroups according to cultural and pathological characteristics: AG 1 includes 3 subgroups; AG 2-2, two subgroups; AG 4, two subgroups, etc. The genetical relationship between AGs and their subgroups was ascertained by DNA base

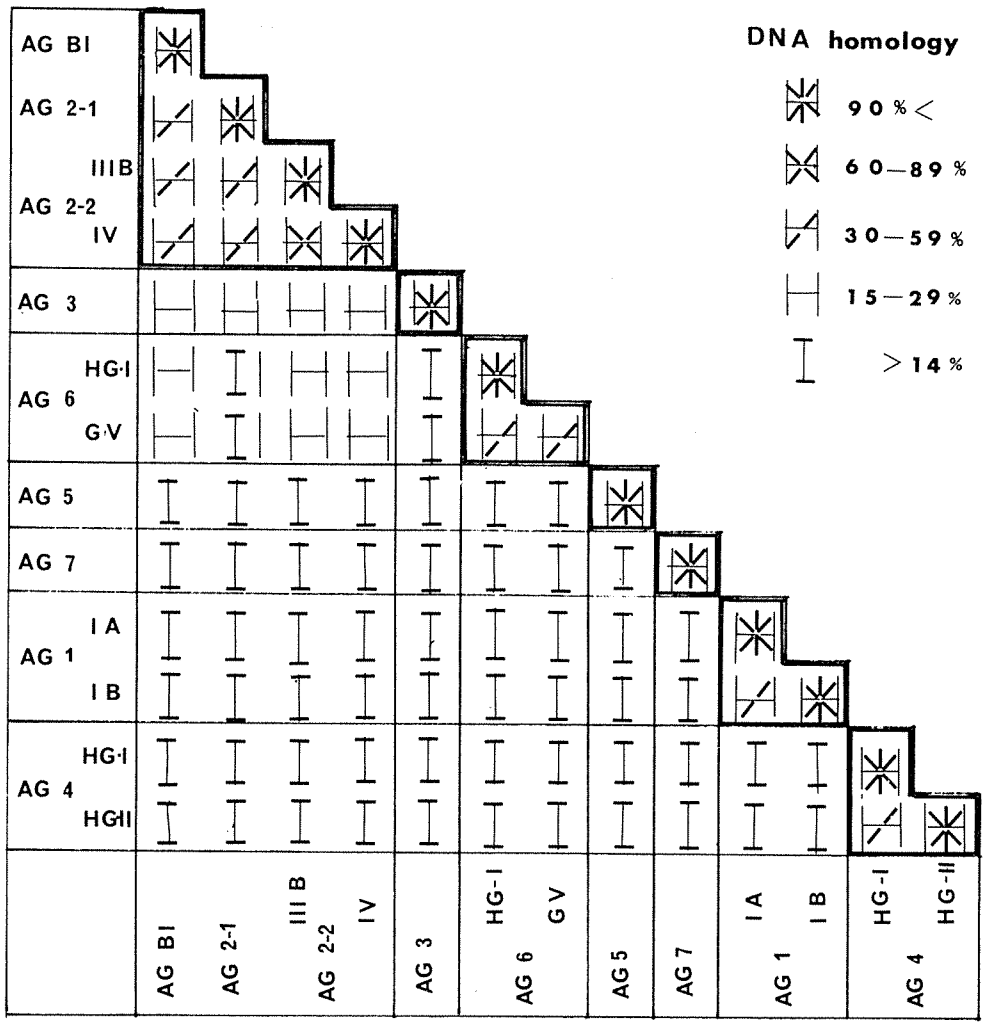

Fig. 1. DNA base sequence homology in the anastomosis groups (AGs) of *R. solani* (Courtesy of S. Kuninaga)

sequence homology[10] (Fig. 1).

AGs in Hokkaido: Anastomosis test of *R. solani* obtained from reserved forests and uncultivated areas in Hokkaido revealed that AG 1, AG 2, AG 3, AG 5, AG 6, and AG BI were native inhabitants of Hokkaido. There are several AGs in cultivated soil and the dominant AG varies with the crop cultivated: i.e., the dominant one in bean field is AG 5 and in sugar beet field AG 2-2. When sugar beet is cultivated for years under monoculture, the diversity distribution pattern of AGs in soil is gradually simplifyed and eventually almost all the isolates in soil belong to AG-2-2. During the monoculture of sugar beet usually the root rot declines gradually.

Rhizoctonia diseases of sugar beet and pathogenic AGs

Sugar beet can be infected with *R. solani* at the different stages of its growth, and various symptoms are

observed: damping-off in seedling stage, root rot or dry rot canker in big tap root observed in the mid-summer, and occasional root rot of stecklings in the propagation farms. Foliage blight appears in the summer when the temperature and humidity are high.

Several AGs were isolated from diseased seedlings grown in paper pots, and they were AG 1-B, AG 2-2, AG 4, AG 5. Binucleate *Rhizoctonia* (*Ceratobasidium* spp.) was most frequently isolated

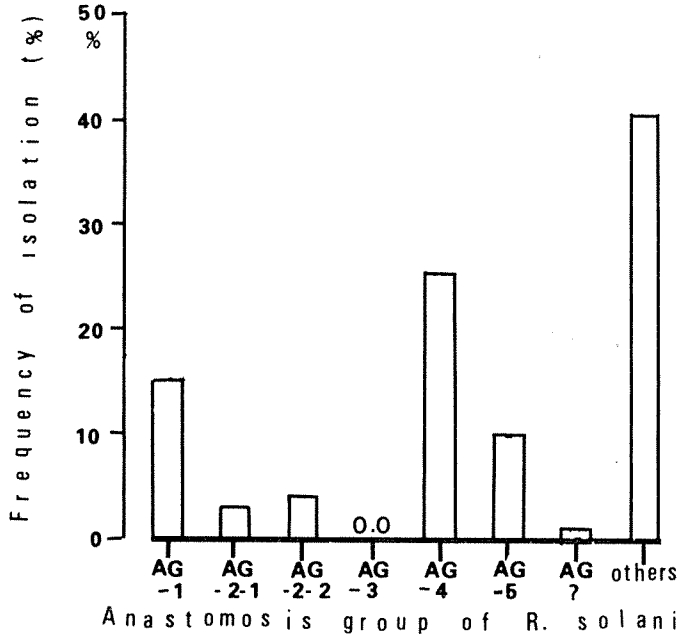

Fig. 2. Frequency of AGs isolated from sugar beet seedlings grown in paper pot. Others=binucleate Rhizoctonia

(Fig. 2). Among them, AG 1-B, AG 2-2, and AG 4 are most virulent, while AG 2-2 was isolated infrequently and caused occasional damping-off in the seedlings grown in paper pots. Paper pots infested with AG 2-2 should not be transplanted, because they constitute inoculum sources for root diseases in the summer, From these results it is concluded that the pathogenic AGs causing damping-off are mainly AG 4 and AG 1-B.

From the crown and tap roots of sugar beet in various fields, AG 2-2 was most frequently isolated, and AG 1-B which caused root rot of steckling was rarely encountered. The inoculation experiments with different AGs in sugar beet fields, the typical sympotms of root rot were induced by AG 2-2. The most suitable method of inoculation was the application of dried grain inoculum of AG 2-2 to soil surface within 3 to 5 cm from the crown, between mid June to early July[14]. Infection of tap roots was caused by the hyphae growing out from inoculum infecting petioles or crown.

After the roots were infected, sclerotia were produced on their surface and in soil around diseased roots. By the sieving-floatation method, 14.3-25.0 and 8.3-10.0 sclerotia/g dry soil were detected

from rhizosphere and non-rhizosphere soils. Generally, more than 80% of sclerotia were in the upper 10 cm of soil and within 10 cm from the diseased root[15].

The germination rate of sclerotia extracted from soil of diseased field was 83% at harvest time, and was 27.6% in the following spring[2]. AG 2-2 was also isolated from particulate plant debris in soil from same field. The average of isolation rate was as high as 35% at harvest time, and extremely low at 1.7% in the following spring. These results pointed out that sclerotia of AG 2-2 which survive in soil are the main inoculum of the following year. Pathogenicity of AG 4, occasionally isolated from soil, was limited to the juvenile tissues of sugar beet.

Basidiospores of AG 2-2 are produced on the surface of petioles and soil, when disseminated, they infect leaf blades, causing small primary lesions. From the lesions hyphae are growing out and invade the epidermis of leaves leading to the appearance of typical symptoms of foliage blight in August when the temperature and air humidity are high. Occasionally AG 1-B was isolated from the primary lesions, only in the early stage of disease.

Therefore, in controlling Rhizoctonia disease of sugar beet, the target AGs are AG 4 in the case of damping-off of seedlings and AG 2-2 in both root rot and foliage blight.

Fungicides for the control of Rhizoctonia diseases

The earliest records of Rhizoctonia diseases are demping-off of eggplants in 1903 and black scurf of potato in 1902 from Hokkaido. Since then, there were many experiments on the control of the diseases, however few of recommended methods were practised. PCNB was the first fungicide introduced to Japan being effective against Rhizoctonia diseases, and it was registered in 1961.

Several anti-Rhizoctonia fungicides were developed in the 1960s, after the outbreak of epidemics of rice sheath blight caused by *R. solani* AG 1. They were organic arsenic compounds and antibiotics. Among them, validamycin A was registered for soil-borne Rhizoctonia diseases, and it is recommended for bottom rot of lettuce, sore-shin of tobacco, and for seed potato treatment. However, it was not effective enough to control black scurf of potato in some fields in Hokkaido where abundant inocula remaind in soil.

There are many fungicides with broad fungicidal spectra, registered for the control of seedling damping-off. Generally, the seed furrow application of these fungicides is effective when the plants are young. Recently, fungicides which are effective against diseases caused by pathogenic Basidiomycetes have been developed. Among them, mepronil was registered for control of damping-off of vegetables, foliage blight and root rot of sugar beet, etc. Iprodione, a fungicide especially effective against Sclerotinia and Botrytis diseases, was registered for controlling Rhizoctonia foliage blight of sugar beet.

Chemical control of Rhizoctonia diseases of sugar beet

Since the paper pot transplanting method in sugar beet cultivation was

Table 2. Activity of fungicides damping-off of sugar beet caused by four AGs of *R. solani* (source: Ass. Hokkaido Sugar Beet Tech., 1978)

Fungicide (conc.)[1]	Incidence of seedling damping-off(%)			
	AG 1[2]	AG 2-2	AG 4	AG 5
hymexazol (40 ppm)	72.4	30.9	100.0	88.4
tolclofos-methyl (40 ppm)	6.1	1.0	1.1	1.1
PCNB (40 ppm)	79.4	27.8	46.7	19.7
control 0	87.3	55.6	98.3	84.8

1) concentration in paper pot soil

2) AGs were inoculated to soil in paper pot

developed in 1957, its use has been increased year by year and now more than 95% of beet growers are using this method in Hokkaido. The method was developed for the purpose of extending the vegetation period and increasing the sugar yield, it also proved to be an excellent and promising method in disease control. One example is its effectiveness in the control of northern root-knot nematode (*Meloidogyne hapla*) once a serious problem in several localities in Hokkaido. The nematode is now scarcely found, because infestation has been prevented in paper pots filled with nematode-free soil. Also damping-off due to either *Pythium* spp. or *Aphanomyces cochlioides* is now so serious as in direct sown sugar beet, if the soil moisture in paper pots is adequately controlled.

The main principle in preventing damping-off in paper pots is to use the pathogen-free or sterilized soil. However, when the paper pots are filled with steamed or fumigated soil with a pH below 5.5, *Trichoderma* sp. vigorously proliferates utilizing cellulose of pot walls, and causes severe seedling blight[6], The adjustment of soil acidity is not recommended, because infection of *Polymyxa betae*, the vector of BNYVV (Rhizomania virus, a virus causing extreme decrease in sugar yield) is suppressed in soil below pH 5.5. From these points chemical control is an inevitable practice in the growth of sugar beet seedlings.

The registered fungicides in soil treatment of paper pots are PCNB for *R. solani*, hymexazole (Tachigaren for *Pythium* spp. and *Aphanomyces cochlioides*. Formerly, they were mixed with soil in advance, but now they were applied in the surface layer of paper pot soil, as Tokkiban (teat plate) was used in seeding operation as shown in the following figure. Thus, the amount of funicides applied is reduced: 50g of PCNB (5% dust) and hymexazole (4% dust) are uniformly mixed with 10 *l* of sieved soil for 8,400 paper pots for a field of 10 a. After seed germination, drench of soil with hymexazol solution is recommended.

In the test of anti-Rhizoctonia fungicides, there are several chemicals to

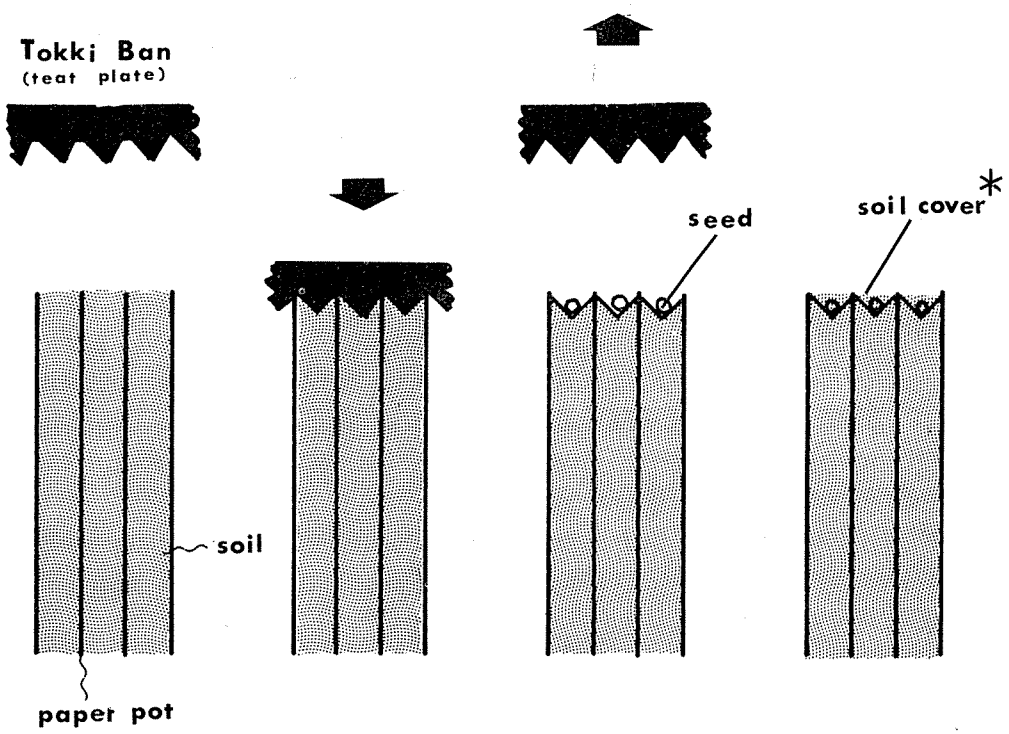

Fig. 3. Application of fungicides into the soil cover of paper pot. Soil cover, mixed with fungicides PCNB (5% dust) 50g and hymexazol (4% dust) 50g were mixed with 10 *l*. of sieved soil. PCNB will be replaced by tolclofos-methyl.

Table 3. Control of root rot by fungicide spray[1)] (source: Ass. Hokkaido Sugar Beet Tech. 1982)

Fungicides[2)]	Root rot (index)[1)]			Root yield (t/ha)
	July 14	Aug. 12	Sept. 10	
PCNB (X 500)	0.19	0.69	1.01	58.3
mepronil (X 500)	0.22	1.25	1.95	48.4
tolclofos-methyl (X 1000)	0.16	0.42	1.18	56.5
control (0)	0.90	3.69	4.12	10.9

1) The test in the field: paper pot grown sugar beet seedlings were transplanted at the end of April, and inoculated with AG2-2 June 25, 1982.

2) fungicides solution was sprayed to crown from both sides of the plants. Date of spray: June 30, July 14. Dosage: 200 *l*/10a
PCNB: 75% wetable powder, mepronil (Basitac): 75% W. P., tolclofos methyl (Rizolex): 50% W. P.

replace PCNB which has been found to be slightly phytotoxic to beet seedlnigs. They are mepronil (Basitac) and tolclofos-methyl (Rhizolex). Both are expected to be registered soon.

After the pot grown seedlings are transplanted, spray of funicides, such as PCNB, TPN or mepronil to the crowns is recommended after the middle of July. The spraying should be done before the fungus starts to infect petioles and crowns. Mepronil and tolclofos-methyl also give good control (Table 3).

Control of foliage blight is achieved by the spray of fungicides. Registered ones are mepronil (Basitac) TPN, TPTH, and iprodioine (Rovral), among them mepronil and iprodione are most effective.

In conclusion, the control of diseases by fungicides, together with the increased application of fertilzers, and wide spread of the paper pot transplanting method greatly contribute to the remarkable increase in the yield of sugar beet root and sugar in Hokkaido (Fig. 4).

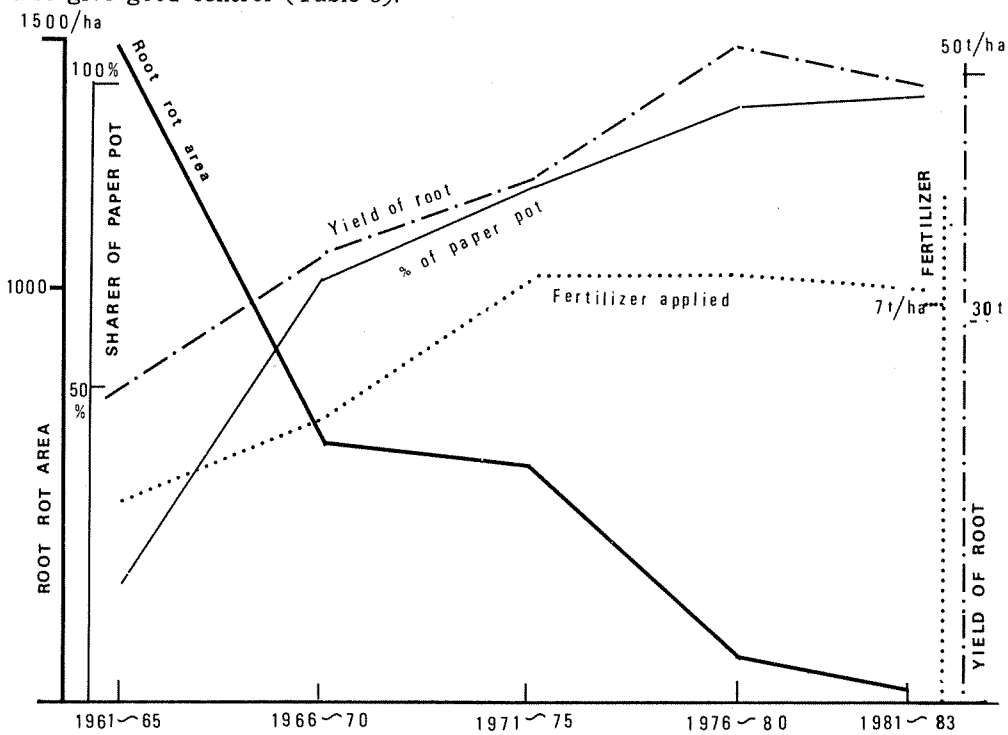

Fig 4. Decreaseing Rhizoctonia root rot and increasing the production of sugar beet by using fungicides, fertilizers and paper pot transplanting method during 1961~1983 in Japan (source; Nippon Beet Sugar Manufacturing Co.).

Prospects of biological control

There have been a number of field trials conducted to control sugar beet root rot by the incorporation of various materials into soil, however, the results have been inconsistant and few of the added materials are suitable for use.

In our experiments, severe root rot resulted from inoculation with AG 2-2 disappeared in the following year in the plants grown in the same plots: disease declined after the initial severe outbreak of root rot.

The decline was found associated with marked reduction of germinable sclerotia, suppression of hyphal growth, and hyphal lysis in soil. Rodshaped bacteria were observed on scleortia and hyphae. Also, diseased isolates of *R. solani*[4] which showed abnormally slow growth in early stage of colony development on agar plate, were obtained from the soil. All of these are believed to be contributing to reduction of inoculum potential of *R. solani* in soil[3].

Reinoculation with AG 2-2 in the following year to the declined plots caused little root rot: after decline, the soil became suppressive to the disease (Table 4). Heat treatemnt of the soil at 100°C for 30 min. eliminated suppressiveness of soil, at 80°C diminished suppressiveness was reduced to a lower level and at 60°C the treatment did not produce any effect. Also, drench of soil

Table 4. Reinoculation of declined soil with *R. solani* in fields receiving inoculation with same fungus previous year (in Experimental Farm in Obihiro[1])

Year of inoculation			Root rot (index) in		
1980	1981	1982	1980	1981	1982
R	R	R	4.28	1.38	2.31
R	O	O	4.38	1.10	0.05
O	R	O	0.10	3.94	0.71
O	O	R	0.09	0.01	3.63

R: inoculated with *R. solani* AG2-2 in the middle of June
O: not inoculated
— root rot index in the year of inoculation
1) sugar beet was monocultured during the experiments

Table 5. Suppressive effect of declined soil in paper pots[1]

Soil in paper pot	Inoculation after transplanting[2]	Root rot index				Yield[3]
		19/VI	16/VII	13/VIII	9/IX	
declined soil	−	0.00	0.01	0.01	0.01	59.3
	+	0.00	1.54	3.62	3.98	20.4
non-declined soil	−	0.00	0.00	0.00	0.08	57.6
	+	0.00	2.23	4.36	4.57	9.0
						LSD 3.9 (1%)

1) Sugar beet was grown in the paper pot filled with declined or nondeclined soil and transplanted into a field and inoculated with AG 2-2.
2) Date of inoculation: June 25
3) t/ha

with streptmycin (2,000 ppm) diminished it partially. From these resullts, soil sppressiveness is thought to be due to multiplication of nonspecific antagonistic microorganisms following the increased population of *R. solani*, especially of the sclerotia produced around severely diseased roots[2].

In the next experiment, sugar grown in paper pot filled with disease declined soil, and the plants were inoculated after they were transplanted into a field (Table 5). Root rot was slightly suppressed and yield of root was significantly high, but suppressive effect was not enough. Efforts are being made to increase the suppressiveness of paper pot soil for achieving improved disease control.

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